Volume 16 Number 12 1988 Nucleic Acids Research

Characterization of response elements for androgens, glucocorticoids and progestins in mouse mammary tumour virus

Jonathan Ham, Axel Thomson, Maurice Needham, Paul Webb and Malcolm Parker*

Molecular Endocrinology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK

Received April 13, 1988; Revised and Accepted May 19, 1988

ABSTRACT

We have characterized steroid response elements in mouse mammary tumour virus (MMTV) by transient transfection. Four partial inverted repeats of the sequence TGTTCT function as response elements for androgen, as well as for glucocorticoid and progestins, although the relative hormone inductions mediated by each oligonucleotide were different. Mutational analysis of the left half of the palindrome showed that a perfect dyad symmetry is not required for optimum activity as a steroid response element. To investigate potential interactions between steroid receptors and transcription factors we have analysed the minimum sequence requirements for a hormone response. Interestingly, a single 15 bp steroid response element and a TATA box are sufficient for steroid inductions. When the distance between the two elements was increased by up to two turns of the helix the hormone induction initially increased and then gradually declined with no obvious periodicity.

INTRODUCTION

Steroid hormones regulate transcription by binding to specific intracellular receptors which interact with steroid response elements, cis-acting DNA sequences that have the properties of transcriptional enhancers (1). We have been investigating such elements in the long terminal repeat (LTR) of mouse mammary tumour virus (MMTV) whose expression is stimulated by several classes of steroid hormone, including glucocorticoids (1 and references therein), androgens (2,3), progestins (4) and mineralocorticoids (5). The possibility that different steroids may act through similar response elements was suggested by the observation that glucocorticoid receptor and progesterone receptor both bound to the same two regions of the MMTV LTR, although the footprints they produced were not identical (6). Both receptor binding sites contained the sequence TGTTCT, one such sequence in the promoter distal binding site and three copies in the proximal site (Fig.1). The LTR sequences bound by other steroid receptors have not yet been mapped but it has been shown by deletion analysis that the androgen receptor functions through the same region as the glucocorticoid and progesterone receptors (7,8). From DNA

sequence comparisons it was evident that the sequence TGTTCT is highly conserved in glucocorticoid response elements and it has been proposed that it forms part of an imperfect palindrome of 15 base pairs (9). Evidence that such a sequence is sufficient as a response element was obtained by showing that a 15 bp oligonucleotide based on a glucocorticoid response element present in the tyrosine amino transferase (TAT) gene was able to confer glucocorticoid inducibility on a heterologous promoter (10). This and similar oligomers were also able to function as progestin response elements (11). To investigate the multihormone control of MMTV expression we have been analysing the function of the four imperfect palindromes present in the region of the LTR which is bound by glucocorticoid and progesterone receptors. Here we show that sequences as short as 15 bp can confer stimulation by androgens as well as glucocorticoids and progestins. However, the four different TGTTCT inverted repeat sequences differ in their ability to function as response elements and show different patterns of induction with the three classes of steroid hormone. We also demonstrate that promoter activity in constructs containing a single 15 bp steroid response element and a TATA box is stimulated by all three classes of steroid and have examined the effect of altering the distance between these two elements.

MATERIALS AND METHODS

Plasmid constructions

Oligonucleotides containing sequences present in the MMTV LTR were cloned into the Xba1 site of pBLCAT2 (12) which contains the thymidine kinase (TK) promoter from herpes simplex virus (13) linked to the gene encoding chloramphenicolacetyl transferase (CAT; 14). For example, the 15 bp inverted repeat centred on position -177 of the LTR was synthesized as two complementary oligonucleotides (5'-CTAGAGTTACAAACTGTT-3' and 5'-CTAGAACAGTTTGTAACT-3'). These were mixed in equal quantities, phosphorylated and heated to 65°C for 30 minutes. After cooling 0.1 ng of the annealed oligonucleotides were ligated with 20 ng of Xba1 digested pBLCAT2. The resulting recombinants were sequenced by the chain termination method (15). A Hinf1 fragment containing the whole MMTV hormone response element was subcloned into the Xba1 site of pBLCAT2 after repair with Klenow DNA polymerase and ligation to Xba1 linkers.

pTK39CAT contains a truncated TK promoter that extends to only 39 bases upstream of the transcription initiation site and was derived from pBLCAT2 as follows. The 163 bp BamH1-Bg111 fragment that contains TK sequences from -105 to +51 bp in relation to the cap site was exchanged for a 90 bp BamH1-Bg111

fragment that extends from -39 to +51 bp. The latter was cut from the HSV linker scanning mutant LS-42/-32 (13).

In pSRETK39CAT the 15 bp steroid response element, centred on position -177 of the MMTV LTR, is positioned 16 bp upstream of the TK TATA box. A series of plasmids in which the response element was moved up to 20 bp further away from the TATA box in 2 bp steps was constructed as follows. Oligonucleotides containing the -177 SRE and 2-20 bp of spacer sequence were cloned as Xba1-BamH1 fragments into pTK39CAT cut with Xba1 and BamH1. Spacer sequences were based on the TK linker scanning mutant LS-56/-46 (13) in which the promoter proximal SP1 binding site was mutated.

Cell culture and transient transfection

ZR-75-1 cells were routinely cultured in DMEM containing 10% foetal calf serum (Gibco, Grand Island, N.Y.) and 10^{-8}M oestradiol. Transient transfections were performed by the calcium phosphate precipitation technique (16). Prior to transfection cells were seeded at a density of 10^6 per 5 cm dish in DMEM containing 3% dextran-charcoal treated serum and 10^{-8}M oestradiol. After 24 hr cells were refed with 4 ml of medium per dish, and then 0.5 ml of precipitate containing 10 µg of plasmid DNA was added. After 6 hr the cells were washed three times with DMEM and were left overnight in medium containing 3% charcoal-treated serum, 10^{-8}M oestradiol. The next day the cells were refed with fresh medium containing appropriate hormones. R5020 and dexamethasone were added to 10^{-7}M and testosterone was used at 2.5 x 10^{-8}M. After 48 hr cells were harvested for CAT analysis and extracts were prepared as reported previously (17). CAT was assayed nonchromatographically according to the method of Sleigh (18). The amount of protein in cell extracts was determined by using a dye-binding assay (Bio-Rad Laboratories). All transfections were performed in duplicate and each experiment was repeated at least twice.

Isolation of total cellular RNA and transcript mapping

To prepare total RNA, cells from a 14 cm diameter petri dish were rinsed twice with phosphate buffered saline and scraped off in 5 ml of the same buffer. The cells were spun down and resuspended in 3 ml of ice-cold NTE (0.1M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) containing 0.5% Nonidet P40 (BDH Chemicals Ltd., Poole, England) and incubated on ice for 10 min. The lysed cells were spun at 5,000 rpm for 5 min and the supernatant extracted once with phenol, twice with phenol/chloroform and once with chloroform alone. The final aqueous layer was made 0.3M with sodium acetate pH 5.5 and two volumes of ethanol were added. After precipitation at -20°C

the RNA was spun down, washed with 70% ethanol and dissolved in 10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 0.1% SDS and stored at -70°C.

Levels of TK CAT RNA were analysed by RNase protection (19,20) using the pSPTKCAT probe constructed by Miksicek et al. (21). Briefly, 20 µg of total RNA and 10^6 dpm of uniformly labelled antisense RNA probe were coprecipitated and redissolved in 30 µl of hybridization buffer (80% formamide, 40 mM PIPES, pH 6.7, 0.4M NaCl and 1 mM EDTA). The samples were heated at 85°C for 20 min and then incubated overnight at 45°C. Unhybridized probe and excess RNA were digested with RNase A and RNase T1 at 20 µg/ml and 1 µg/ml respectively in 10 mM Tris-HCl, pH 7.5, 5 mM EDTA and 0.3M NaCl. After proteinase K digestion and phenol extraction the RNase resistant products were recovered by precipitation and separated by electrophoresis on 6% acrylamide, 8.3M urea sequencing gels.

Steroid hormone receptor binding assays

Cytoplasmic steroid receptor concentrations were determined by the dextran-coated charcoal procedure (22) by competition-binding assays on cytosol preparations with the use of single saturating concentrations of radio-labelled ligand.

RESULTS

A 15 bp sequence can function as a steroid response element for androgens, glucocorticoids and progestins

The function of the distal and proximal binding sites for glucocorticoid and progesterone receptors in MMTV has been analysed by transient transfection in the human breast cancer cell line ZR-75-1 which contains receptors for androgen (588 fmoles per mg cytoplasmic protein), glucocorticoid (151 fmoles per mg protein), oestrogen (73 fmoles per mg protein) and progestins (177 fmoles per mg protein). The two receptor binding sites together contain four copies of the sequence TGTTCT each of which forms part of a 15 bp partial dyad symmetry (6; Fig.1). The function of each 15 bp sequence has been compared with that of the whole region when cloned upstream of the TK promoter fused to the chloramphenicol acetyl transferase (CAT) gene in pBLCAT2 (12). A DNA fragment extending from -469 to -69 bp upstream of the MMTV cap site contains both the distal and proximal binding sites and functions as a response element for androgens as well as for glucocorticoids and progestins, irrespective of its orientation (Fig.2). A 15 bp oligonucleotide based on the partial palindrome centred on position -177 (contained within the distal binding site) had similar properties. We next

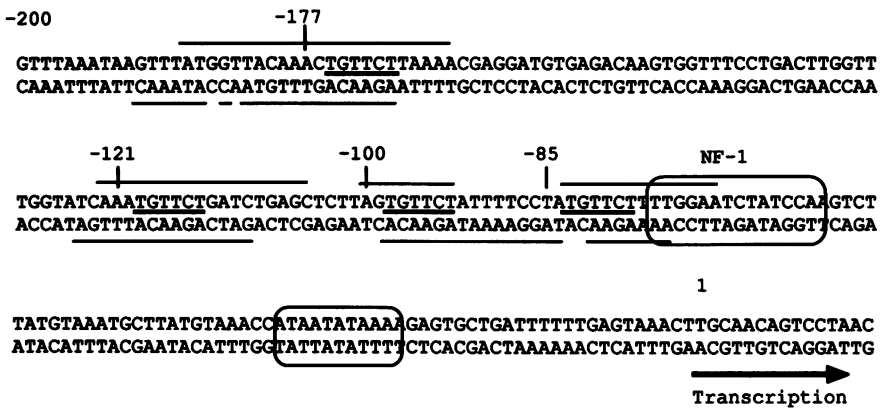

Fig.1 Regulatory Elements in the LTR of MMTV
The distal (-190 to -160) and proximal (-120 to -70) binding sites for glucocorticoid receptor are over- and underlined and the four TGTTCT sequences are indicated. An NF-1 binding site and TATA sequence are boxed. The start of transcription is shown and numbering is in relation to this position.

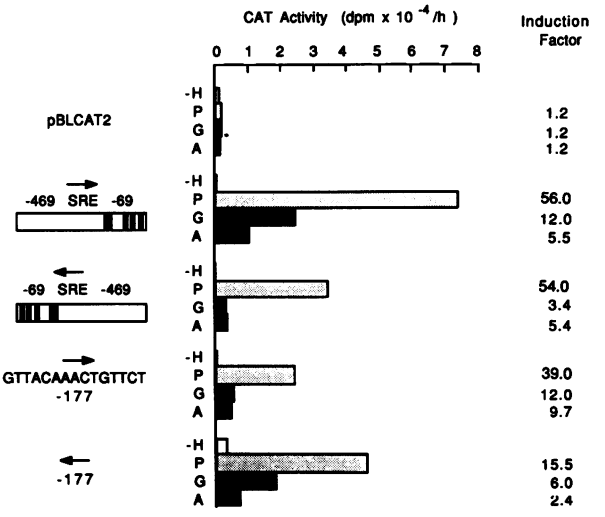

Fig. 2 DNA Sequence Requirements for Steroid Response Element Activity
MMTV DNA -469 to -69 or an oligonucleotide containing the inverted repeat GTTACAAACTGTTCT centred on position -177 of the LTR were subcloned into the Xba1 site of pBLCAT2 (12) and transiently expressed in ZR-75-1 cells. Transfected cells were grown in the absence (-H) or presence of 2.5×10^{-8} M testosterone (A), 10^{-7} M dexamethasone (G) or 10^{-7} M R5020 (P) for 48 hr and equal quantities of extract were assayed for CAT activity as described in the Methods section. The induction factor for each steroid is shown on the right-hand side. The relative orientation of the whole steroid response element (SRE) and of the -177 oligonucleotide is shown by arrows. The position of the four TGTTCT inverted repeats within the SRE are indicated by solid blocks.

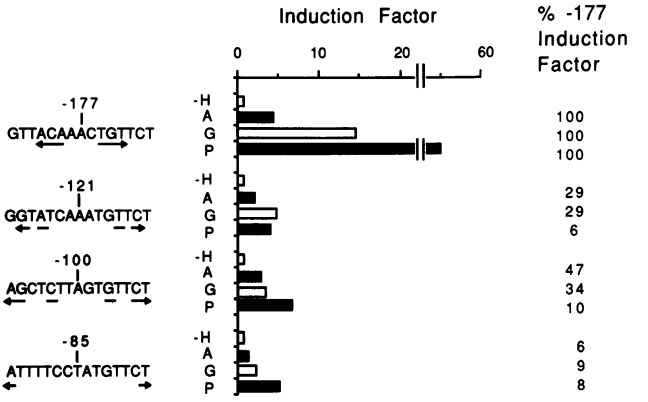

Fig.3 Comparison of MMTV oligomers as Steroid Response Elements
Oligomers based on the four different TGTTCT inverted repeats centred on positions -177, -121, -100 and -85 of the MMTV LTR were compared for their activity as steroid response elements. Induction factors for testosterone (A), dexamethasone (G) and R5020 (P) are shown together with the % induction conferred by each oligomer relative to that of the -177 oligomer.

examined the function of the other three TGTTCT inverted repeats within the proximal binding site and found that they too were capable of conferring androgen, glucocorticoid and progestin responses on the TK promoter although the inductions were smaller than those for the -177 element (Fig.3). The relative inductions by each steroid differed for each oligonucleotide. For example, the progestin responses conferred by the -121 and -100 elements were appreciably less than that of the -177 element (6% and 10%) while their activity as androgen and glucocorticoid response elements was only moderately reduced (29%-47%). These results suggest that the relative affinities of the three hormone receptors were slightly different for the four TGTTCT elements.

To confirm that the changes in CAT activity observed in these experiments were due to alterations in the steady state level of CAT mRNA, ZR-75-1 cells were transiently transfected with the -177 oligonucleotide construct and total RNA was isolated after 48 hr of hormone treatment. Samples were hybridized to a uniformly labelled TKCAT antisense RNA probe, digested with RNAses A and T1 and the products were resolved on a sequencing type gel (Fig.4). Correct initiation from the TK promoter would be expected to result in a protected fragment of 210 bases (indicated by an arrow). In an experiment in which CAT activities were stimulated approximately 30-fold, 4-fold and 8-fold by R5020, dexamethasone and testosterone respectively,

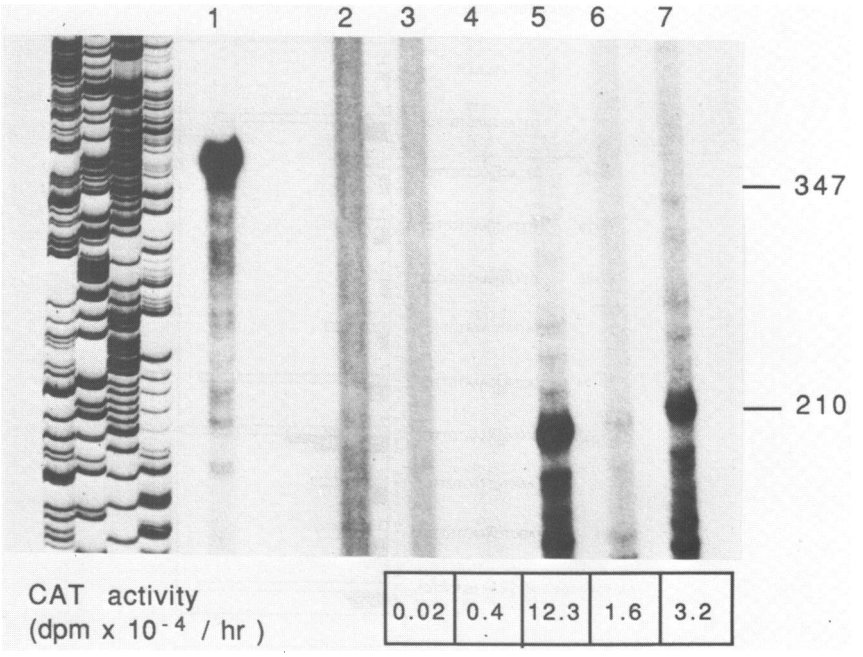

Fig.4 Effect of Steroids on CAT activity and TKCAT mRNA concentration
pBLCAT2 containing the -177 response element was transiently transfected into ZR-75-1 cells and TKCAT mRNA concentrations determined by RNase protection were compared with CAT activity. A DNA sequence ladder (GATC) was run as a set of molecular weight markers and the positions of the probe (347 nucleotides) and of the protected fragment derived from correctly initiated TKCAT RNA (210 nucleotides) are shown by arrows on the right-hand side. The untreated antisense RNA probe was loaded in lane 1. Lanes 2-7 contain the products of hybridizations with 20 µg of tRNA (lane 2) or 20 µg total RNA from cells that were mock transfected (lane 3) or transfected with the -177 oligonucleotide construct and treated with no hormone (lane 4), 10^{-7}M R5020 (lane 5), 10^{-7}M dexamethasone (lane 6) or 2.5 x 10^{-8}M, testosterone (lane 7). The CAT activities determined in a portion of the cells harvested from each dish are shown beneath lanes 3-7.

changes in the level of the protected fragment paralleled the increases in CAT activity. Thus changes in CAT activity result from changes in mRNA abundance.

Point mutations within steroid response elements can destroy or improve their function

Although all four of the 15 bp MMTV steroid response elements contain the sequence TGTTCT none of them show perfect dyad symmery (see Fig.3). The two most palindromic sequences (those centred on positions -177 and -100 in the LTR) both contain 3 bases in each half of the inverted repeat that are complementary, yet the -177 sequences (GTTACAAACTGTTCT) is a more active

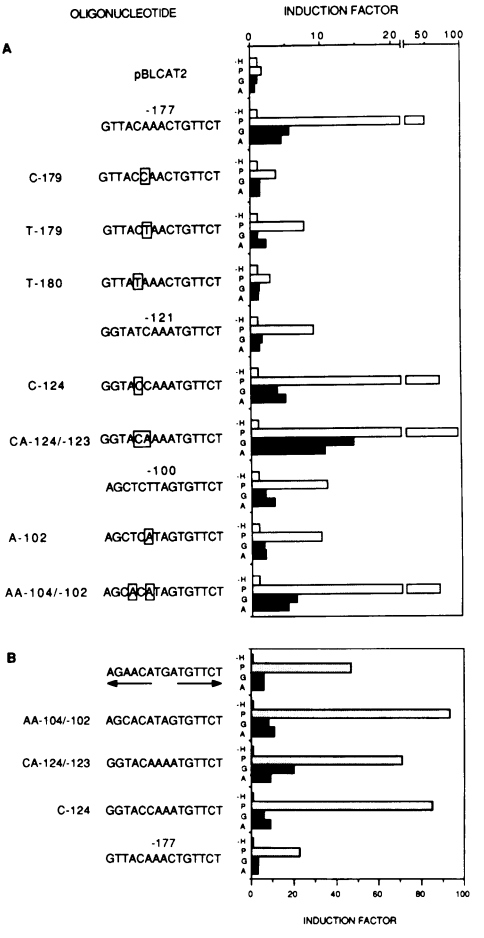

Fig.5 Effect of Point Mutations on Steroid Response Element Activity
pBLCAT2 containing the -177, -121 and -100 oligomers or mutant variants of these sequences were transiently transfected into ZR-75-1 cells and the inductions produced by R5020 (P), dexamethasone (G) and testosterone (A) were determined. The point mutations in each oligomer are boxed and their sequence designation is shown on the left-hand side.

response element than the -100 (AGCTCTTAGTGTTCT). This prompted us to test whether the sequence ACA, which is present in the left half of the -177 palindrome and complementary to the first three bases of the TGTTCT motif, is important for the greater activity of this response element. Three different point mutations were introduced into this sequence (Fig.5A). Two changed the final A of ACA and one changed the central C. All three changes greatly reduced inductions with all three classs of hormone. Conversely we also

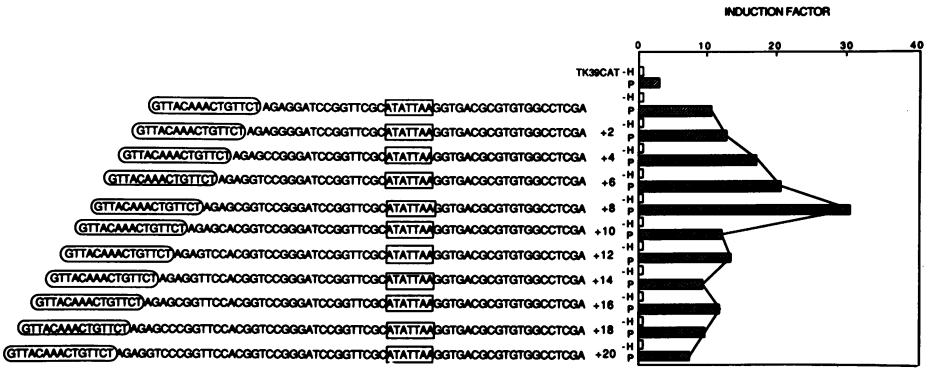

Fig.6 Effect of Alterations in the Spacing between the -177 Response Element and a TATA box

The effect of R5020 on CAT activity was determined for a series of constructs in which the -177 response element was moved from 16 to 36 bp away from the TATA box. The spacer sequence was based on the wild type TK promoter.

mutated the -121 and -100 response elements one bp at a time into variants that contained the sequence ACA (Fig.5A). Three such changes, C-124, CA-124/-123 and AA-104/-102 significantly increased hormone inductions confirming that the sequence ACA is important for the high activity of the -177 response element. However, by comparing the highly active response element C-124 (GGTACCAAATGTTCT) and the weak response element C-179 (GTTACCAACTGTTCT) it is apparent that the single difference GGT→GTT also leads to a substantial change in activity. Furthermore, during the course of these experiments it became clear that the mutant oligonucleotides C-124, CA-124/-123 and AA-104/-102 gave greater hormone inductions than a perfect inverted repeat of the sequence TGTTCT (Fig.5B). Therefore a perfect palindrome is not required for optimum activity as a steroid response element.

A 15 bp steroid response element and a TATA box are sufficient for hormonal regulation of transcription

So far we have shown that 15 bp oligonucleotides based on partial inverted repeats of the sequence TGTTCT function as steroid response elements that modulate the activity of the TK promoter. This promoter has been shown to contain binding sites for three different transcription factors, two GC boxes recognized by SP1, a CAAT box and a TATA box. We have tested whether the -177 oligonucleotide could stimulate the activity of the TK TATA box element alone. When the steroid response element was placed 16 bp upstream of the TATA box the basal level of CAT activity was less than that obtained with the

whole TK promoter but induction by androgens, glucocorticoids and progestins was maintained (data not shown). The control vector TK39CAT shows a small stimulation of CAT activity by progestin but this is unlikely to be a specific effect because this vector lacks a steroid response element (Fig.6). To investigate whether the stereospecific alignment of the two elements could affect the hormone responses observed, a series of constructs was made in which the response element was moved from 16 bp to 36 bp away from the TATA box in 2 bp steps. The spacer sequences used were based on the linker scanning mutant LS-54/-46 (Fig.6). Basal levels of CAT activity were similar for all of these constructions (data not shown) but progestin inductions varied (Fig.6). As the SRE is moved away from the TATA box the induction factor initially increases reaching a maximum when the two elements are separated by about 3 turns of the DNA helix and then gradually declines. However, over the distance tested we did not observe a 10 bp periodicity that would reflect the relative alignment of the two elements on the surface of the DNA helix.

DISCUSSION

We have demonstrated that a 15 bp oligonucleotide containing an imperfect inverted repeat of the sequence TGTTCT can function as a response element for androgen as well as for progestin and glucocorticoid. This observation implies that the DNA binding domain of the androgen receptor may have an amino acid sequence closely related to those of the progesterone and glucocorticoid receptors, which show 91% identity at the amino acid level (23, and references therein). Since the human mineralocorticoid and glucocorticoid receptors show 94% identity in their DNA binding domains (5) it is likely that mineralocorticoid can also act through the same DNA sequences.

By comparing the four different MMTV inverted repeats and mutant variants of these in a single cell line that expresses receptors for androgens, glucocorticoids and progestins we have been able to detect slight differences in activity. This suggests that although androgen, glucocorticoid and progesterone receptors recognize a response element distinct from that for oestrogens (10,24) the three receptors do have slightly different sequence specificities. Interestingly the hormone inductions we have observed do not reflect the relative receptor concentrations in ZR-75-1 cells as assayed by ligand binding. For example, while the progesterone receptor concentration is one-third that of the androgen receptor all of the oligonucleotides tested gave a greater progesterone response; this may be a consequence of a

difference in affinity of the two receptors for their response element. If at least three different classes of steroid hormone can act through the same element, how could genes that contain such sequences be specifically regulated by one particular steroid *in vivo*? Differences in the relative abundance of different classes of receptor in combination with small sequence specific differences in steroid responsive activity can only partly explain this paradox and it is likely that other factors are important. For example, additional proteins may be involved so that perhaps each class of steroid receptor differs in its ability to interact with particular positively-acting transcription factors or to counteract the effects of negative regulators of transcription.

The distal and proximal receptor binding sites in the MMTV LTR contain one and three TGTTCT palindromes respectively. We have found that individually all four sequences do not differ by more than three-fold in their ability to function as androgen or glucocorticoid response elements but the repeat centred on position -177 is considerably more active as a progesterone response element than the three others. This result is supported by exonuclease III footprint studies (6) which indicated that progesterone receptor had a relatively greater affinity for the distal binding site than the glucocorticoid receptor. Thus if the individual effects of the four different repeats are additive then the distal palindrome would play a more important rôle than the proximal sequences in the progestin response while all four inverted repeats would contribute approximately equally to the overall androgen or glucocorticoid response mediated by the LTR. However, Martinez *et al*. (24) have shown that two partially palindromic oestrogen response elements in the promoter region of the *Xenopus laevis* vitellogenin B1 gene act together cooperatively whereas the individual elements are inactive. Consequently it is also possible that there may be some cooperative effects involving the response elements in the MMTV LTR.

An attractive model for the mechanism by which transcriptional transactivation occurs proposes that DNA binding proteins involved in gene activation interact directly by protein-protein contacts (25). In terms of steroid hormone action, this would involve an interaction between a steroid receptor complex and a transcription factor. Therefore when the receptors for androgen, glucocorticoid or progesterone stimulate the activity of the TK promoter they could potentially interact with one or more of the three different transcription factors which have binding sites in the promoter, namely SP1, CTF, and the TATA box binding factor. Here we have demonstrated

that androgens, glucocorticoids and progestins can stimulate the activity of a reporter gene when a single 15 bp steroid response element and a TATA box are closely juxtaposed. In terms of a model based on protein-protein interactions this finding suggests that steroid receptors are capable of directly interacting with either the TATA box factor or with RNA polymerase itself. However to date there is no direct evidence to support this model.

By moving the steroid response element away from the TATA box through two turns of the DNA helix we showed that the relative spacing between the two elements affects hormone inducibility. However, we did not observe a pattern of change with a periodicity of 10bp, which would occur if efficient interaction between the two proteins depended on their binding sites lying on the same face of the DNA helix. Such effects have been observed for several procaryotic transcription factors. Cooperative binding of phage λ repressor to adjacent pairs of operator sites has been shown to occur only when the two sites are located on the same side of the DNA helix (26). Interaction between repressors bound to operators separated by several helix turns was proposed to cause looping of the intervening DNA. Such loops were subsequently visualized by electron microscopy (27). The effect of altering the stereo-specific alignment of sequences bound by transcriptional regulatory proteins has also been investigated in several eukaryotic genes. For example, cyclical changes in transcriptional activity with a periodicity of 10 bp were observed when elements of the SV40 early promoter were moved relative to each other (28) and when the distance between a steroid response element and CACCC box was altered (29). Furthermore, Théveny et al. (30) showed by electron microscopy that DNA loops could form between progesterone receptors bound to response elements at a distance from one another suggesting that steroid receptors can interact with each other. However, there are examples of other systems where such effects have not been apparent. For example, changes in the stereo-specific alignment of an octamer element and β-globin TATA box (31), or of two oestrogen response elements that acted cooperatively (24) did not lead to alterations in transcriptional activity.

If direct protein-protein interactions between receptor and a TATA box binding factor do occur why were changes in transcriptional activity with a 10 bp periodicity not observed when the stereospecific alignment of the two elements was altered? It is possible that the steroid-receptor complex bound to its response element and/or the TATA box binding factor may be sufficiently large or flexible enough to interact effectively when the binding sites for the two proteins are on opposite surfaces of the DNA helix.

Alternatively, the receptor and TATA box factor might interact independently with RNA polymerase II. We would then predict that steroid hormones could stimulate transcription through a single steroid response element in the absence of a binding site for any other transcription factor.

In most of the steroid responsive promoters characterized so far the binding site(s) for receptor are more than two helix turns from the TATA box. In these situations the receptor may interact with other transcription factors that bind to adjacent sequences. For example, in the MMTV LTR there is an NF-1 binding site located between the four response elements and the TATA box which has been shown to be necessary for the hormone response (32,33). It is conceivable that in genes where the response elements are located far upstream of the start of transcription such as uteroglobin (34) and (TAT) (9) there may be binding sites for transcription factors nearby.

Finally, it is also necessary to consider a number of other possible mechanisms for the action of steroid receptors including effects on DNA structure, DNA supercoiling or the positioning of nucleosomes. In the case of the MMTV LTR it is known that hormone treatment results in alterations in nucleosome phasing over the hormone response element (35) and receptor may stimulate transcription by altering chromatin structure so that transcription factors are able to bind to bind to their target sequences. In experiments to distinguish between the different models for transcriptional activation the simple hormone responsive promoters we have constructed may offer certain advantages over more complex natural promoters.

ACKNOWLEDGEMENTS

We are extremely grateful to I. Goldsmith for providing oligonucleotides, to S.L. McKnight for providing TK linker scanning mutants and to B. Luckow, R. Miksicek and G. Schütz for the gifts of pBLCA2 and pSPTKCAT. We thank S. Goodbourne, R. King and J. Rosen for their comments on the manuscript.

*To whom correspondence should be addressed

REFERENCES

1. Yamamoto, K.R. (1985) Ann. Rev. Genet. 19, 209-252.
2. Darbre, P., Dickson, C., Peters, G., Page, M., Curtis, S. and King, R.J.B. (1983) Nature 303, 431-433.
3. Darbre, P., Page, M. and King, R.J.B. (1986) Mol. Cell. Biol. 6, 2947-2854.
4. Cato, A.C.B., Miksicek, R., Schütz, G., Arneman, J. and Beato, M. (1986) EMBO J. 5, 2237-2240.
5. Arriza, J.L., Weinberger, C., Cerelli, G., Glaser, T.M., Handelin, B.L., Housman, D.E. and Evans, R.M. (1987) Science 237, 268-275.

6. von der Ahe, D., Janich, S., Scheidereit, C., Renkawitz, R., Schütz, G. and Beato, M. (1985) Nature 313, 706-709.
7. Cato, A.C.B., Henderson, D. and Ponta, H. (1987) EMBO J. 6, 363-368.
8. Parker, M.G., Webb, P., Needham, M., White, R. and Ham, J. (1987) J. Cell Biochem. 35, 285-292.
9. Jantzen, H.-M., Strähle, E., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R. and Schütz, G. (1987) Cell 49, 29-38.
10. Klock, G., Strähle, U. and Schütz, G. (1987) Nature 329, 734-736.
11. Strähle, U., Klock, G. and Schütz, G. (1987) Proc. Natl. Acad. Sci. USA 84, 7871-7875.
12. Luckow, B. and Schütz, G. (1987) Nucleic Acids Res. 15, 5490.
13. McKnight, S.L. and Kingsbury, R. (1982) Science 217, 316-324.
14. Gorman, C.M., Moffat, L.F. and Howard, B.H. (1982) Mol. Cell. Biol. 2, 1044-1051.
15. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
16. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. and Chasin, L. (1979) Proc. Natl. Acad. Sci. USA 76, 1373-1376.
17. White, R., Lees, J.A., Needham, M., Ham, J. and Parker, M. (1987) Mol. Endocrinol. 1, 735-744.
18. Sleigh, M.J. (1986) Anal. Biochem. 156, 251-256.
19. Zinn, K., DiMaio, D. and Maniatis, T. (1983) Cell 34, 865-879.
20. Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K. and Green, M.R. (1984) Nucleic Acids Res. 12, 7035-7056.
21. Miksicek, R., Heber, A., Schmid, W., Danesch, U., Posseckert, G., Beato, M. and Schütz, G. (1986) Cell 46, 283-290.
22. Korenman, S.G. (1968) J. Clin. Endocrinol. & Metab. 18, 127-130.
23. Green, S. and Chambon, P. (1986) Nature 324, 615-617.
24. Martinez, E., Givel, F. and Wahli, W. (1987) EMBO J. 6, 3719-3727.
25. Ptashne, M. (1986) Nature 322, 697-701.
26. Hochschild, A. and Ptashne, M. (1986) Cell 44, 681-687.
27. Griffith, J., Hochschild, A. & Ptashne, M. (1986) Nature 322, 750-752.
28. Takahashi, K., Vigneron, M., Matthes, H., Wildeman, A., Zenke, M. and Chambon, P. (1986) Nature 319, 121-126.
29. Schüle, R., Muller, M., Otsuka-Murakami, H. and Renkawitz, R. (1988) Nature 332, 87-90.
30. Théveny, B., Bailly, A., Rach, C., Rach, M., Delain, E. and Milgrom, E. (1987) Nature 329, 79-81.
31. Wirth, T., Staudt, L. and Baltimore, D. (1987) Nature 329, 174-178.
32. Buetti, E. and Kühnel, B. (1986) J. Mol. Biol. 190, 379-389.
33. Miksicek, R., Borgmeyer, U. and Nowock, J. (1987) EMBO J. 6, 1355-1360.
34. Jantzen, K., Fritton, H.P., Igo-Kemenes, T., Espel, E., Janich, S., Cato, A.C.B., Mugele, K. and Beato, M. (1987) Nucleic Acids Res. 15, 4535-4552.
35. Richard-Foy, H. and Hager, G.L. (1987) EMBO J. 6, 2321-2328.